

## First record of Aitengidae (Mollusca: Panpulmonata: Acochlidia) for Australia

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### Abstract

Currently there are only two species of the highly enigmatic family of microscopic 'sea slugs' Aitengidae (Mollusca: Gastropoda, Panpulmonata: Acochlidia) known worldwide: the mangrove-dwelling *Aiteng ater* Swennen & Buatip, 2009 from Thailand and *Aiteng mysticus* Neusser *et al.*, 2011 from the upper intertidal of coastal caves in Japan. Here we report the discovery of another species of Aitengidae found in a mangrove forest in Darwin Harbour, Northern Territory, Australia. This is the first record of the Aitengidae, and the Acochlidia (caddis slugs) in general, for Australia so it represents a very significant finding. In Darwin Harbour the *Aiteng* specimens are diurnal and inhabit areas of the mangrove forest influenced by freshwater. Externally, these Australian specimens differ from the other described species by the presence of a pair of short, but clearly discernible, cephalic tentacles. The first molecular analyses on cytochrome oxidase (COI) sequences show considerable differences to other known Aitengidae. An integrative approach including molecular multimarker analyses and detailed microanatomical and histological investigations needs to be undertaken to reveal the relationships within the Aitengidae.

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The Aitengidae was only recently (2009) established as a monotypic family of sacoglossans (sap-sucking sea slugs), however this relationship was doubted and possible affinities with Acochlidia (caddis slugs) were considered (Swennen & Buatip 2009). Its type species, *Aiteng ater* Swennen & Buatip, 2009, is amphibious and lives high in the intertidal zone on the mud and in small pools in a dense mangrove forest in the Gulf of Thailand. This mysterious 'bug-eating slug' was selected into the Top 10 list of bizarre new animal species 2010 by the International Institute of Species Exploration (IISE 2010).

When they were first discovered in Thailand, aitengids were thought to be juvenile mangrove slugs (Onchidiidae) until the absence of stalked eyes was noted and further

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studies showed they had no relationships with onchidiids at all (Swennen & Buatip 2009). The initial specimens were considered so extraordinary that a new family, Aitengidae, was created for them. The name for the genus itself, *Aiteng*, was chosen from Ai Theng, which is the name of one of the popular puppets in shadow plays (wayang) in the southern part of Thailand. Ai Theng is a jolly, smooth, black male who plays in the night. Extraordinarily for a sea slug, these original specimens of *Aiteng ater* were found to be able to open the pupal cases of beetles and moths living in the mud with them and eat the contents, as well as consume ant larvae and whole adult mosquitoes (Swennen & Buatip 2009).

An integrative approach combining detailed microanatomical 3D-reconstruction based on histological sections and molecular analyses conducted soon after the discovery revealed the true relationship of *Aiteng ater* (Neusser *et al.* 2011). The Aitengidae clusters robustly among hedylopsacean acochlidians and the family was firmly assigned to the Acochlidia (Mollusca: Gastropoda: Panpulmonata) (Neusser *et al.* 2011). At the same time a second, new aitengid species was described. *Aiteng mysticus* Neusser *et al.*, 2011 from Japan occurs on algae in the upper intertidal zone on rocky shores in tiny crevices of small coastal caves. It was also found in a brackish area neighbouring a mangrove forest on the underside of large, wet rocks which are deeply embedded in mud.

Here we present the first record of aitengid specimens from Australia found live during an environmental baseline survey of a mangrove forest fringing Bennetts Creek, a small tributary of the Elizabeth River, located in the East Arm of Darwin Harbour. On 21 March 2012, three animals were discovered in three different floristic assemblages within a localised area of mangrove forest influenced by seasonal freshwater inflow during the wet season (Table 1). The specimens were found in the upper intertidal in the mixed species hinterland margin community, in the tidal flat forests dominated by *Ceriops australis* and in the lower tidal creek community (Figure 1). The latter was dominated by the Stilt-root Mangrove (*Rhizophora stylosa*) and the Kapok Mangrove (*Camptostemon schultzei*) and no rocks were present in the substrate. Three additional animals were collected at this same locality on 5 March 2013 (Table 1).

The habitat throughout this mangrove forest has significant freshwater inflow derived from the paperbark (*Melaleuca viridiflora*) forest to landward. Indeed, there was a permanent spring-fed fresh/brackish pool at the landward edge of the mangrove forest which supplied the collecting site with freshwater sheet-flow during the wet season. The salinity of the habitat was not measured, but based on the combination of seasonal freshwater sheet-flow and tidal activity observed at the site, salinity probably ranged from near fresh during the wet season to a maximum of 36 ppt during the late dry season. Salinities in the Elizabeth River system are seasonally diluted, varying between 20–30 ppt during the wet season (Wrigley *et al.*



**Figures 1-4.** Habitat and living specimens of Australian *Aiteng* sp.: 1. Bennetts Creek in Darwin Harbour – mangrove site showing the *Rhizophora stylosa* dominated forest where the slugs were most commonly found. 2. Individual crawling on the underside of a rotting log on the forest floor. 3. Specimen found on the forest floor, crawling on top of an algal turf mat. 4. Live animal approx. 4 mm (two photos of same animal) showing the distinctive black-glossy notum, conspicuous eye spots and short cephalic tentacles. (Adam Bourke)

1990) and falling as low as 2–18 ppt during peak wet season flows (Drewry *et al.* 2010).

The specimens found in 2012 were collected by Kristin Metcalfe and Adam Bourke, while the specimens found in 2013 were collected by Adam Bourke. All the specimens were found after spring tidal cycles during the monsoon season. No specimens were found during a collection attempt in the wet season of March 2014 during neap tides, or during a single dry season survey undertaken previously in September 2011. All the specimens were found within 6–75 m from the tidal creek at low tide during the day.

**Table 1.** Details of collecting site and specimens of *Aiteng* sp. in Australia.

Station number	Location	Number of specimens	Fixative (ethanol)	Collection date
BC-2	12.58779°S 131.02186°E	1	70%	21 March 2012
BC-2	(as above)	3	100%	5 March 2013
BC-4	12.58764°S 131.02161°E	1	70%	21 March 2012
BC-6	12.58753°S 131.02132°E	1	70%	21 March 2012

Animals were found actively crawling around on the forest floor or hiding within rotting logs. One specimen collected in 2012 measuring 2 mm preserved length was preserved in 70% ethanol and stored in the mollusc collection of the Museum and Art Gallery of the Northern Territory (NTM P.48762). Both remaining specimens collected in 2012 (3.7 and 2.9 mm preserved length) were preserved in 70% ethanol for histological and anatomical investigations. The specimens discovered in 2013 from the same habitat were collected from the undersurface of a fallen dead mangrove log of the Grey Mangrove (*Avicennia marina*) (Figure 2) and from the forest floor in the *Rhizophora stylosa* dominated tidal creek assemblage (Figure 3). One individual measuring 7.3 mm extended crawling length was preserved in 100% ethanol for molecular analyses (Table 1).

In life, the specimens are greater than 7 mm when extended and crawling actively. The dorsal notum is glossy black. Neither the head nor the area around the eyes is pigmented. The foot is cream (Figures 2, 4). The Darwin Harbour specimens have one pair of large broad lappets ventrally above the foot, and in contrast with *A. ater*, have one pair of very short cephalic tentacles (Figure 2–4). The head can be retracted under the frontal notal border in both species.

One of the Darwin Harbour specimens was observed in the field crawling on top of a mat of algal turf. It was possibly feeding on the algae, or on prey present within the algae. When the specimens were subjected to seawater in the laboratory, they retracted their head, suggesting they are intolerant of high salinities. In contrast, animals kept in freshwater displayed normal locomotion and were moving freely around within petri dishes. This is in contrast to observations overseas that individuals usually avoid any direct contact with water (independent of salinity) and prefer a humid environment instead (Timea Neusser, pers. obs.).

In summary, the present specimens resemble other aitengid species in external morphology and habitat. However, dissimilarities regarding the shape and behaviour are evident, and this has been confirmed by molecular analyses on cytochrome oxidase (COI) sequences (Timea Neusser, unpubl. data). A future integrative approach combining a detailed microanatomical study with molecular multimarker analyses will shed light on the relationship of the Australian species with the described aitengid species.

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